SPORULATION AND VIABILITY OF OÖCYSTS OF EIMERIA ARLOINGI FROM THE DOMESTIC SHEEP[1]

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INTRODUCTION

Although a considerable amount of general information is available concerning the factors that influence the sporulation and viability of oöcysts of sheep coccidia, more specific knowledge is needed for a logical approach to the problem of control of coccidiosis through prevention.

Most of the available facts concerning the behavior of oöcysts of ovine coccidia are attributable to the work of Lerche.[2] Other investigators, namely, Douwes,[3] Yakimoff and his colleagues,[4] and Carré,[5] have largely confirmed and expanded the conclusions of Lerche. From the work of these investigators, it is known that the oöcysts never sporulate inside the intestine of the host because of the prevailing anaerobic conditions; that oöcysts discharged from the host in fecal pellets sporulate within 2 to 3 days, provided conditions of temperature, moisture, and oxygen tension are optimum; that excellent conditions for sporulation often occur in the litter of the fold and in protected sites in pasture grass; that putrefaction and drying are destructive to oöcysts, whereas cold retards the rate of sporulation; and that infection of a new host takes place through ingestion of feed and drink contaminated with fecal material containing sporulated oöcysts.

The general nature of these conclusions may be attributed to lack of detailed experimental work in which all factors have been rigidly controlled. A precise knowledge of conditions favorable or inimical to sporulation is desirable in order to facilitate the recognition and elimination of specific locations in pastures and yards that are favorable to the sporulation and preservation of oöcysts. It is also of practical importance to know how long sporulated or unsporulated oöcysts will retain their viability under given conditions. Conclusions derived from data on sporulation and viability experiments constitute the best basis for formulating measures for the prevention and control of coccidiosis.

The present study was undertaken in order to define more accurately the conditions that favor, retard, or prevent sporulation and destroy or preserve viability of the oöcysts. Although the generalizations presented probably apply equally to all species of ovine coccidia, the data were obtained entirely from experiments on oöcysts of *Eimeria arloingi* Marotel, which occur in greater abundance and frequency

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[2] LERCHE, MARTIN. DIE KOKZIDIOSE DER SCHAFE. Deut. Tierärztl. Wchnschr. 28: 489–494. 1920.
———. DIE COCCIDIOSE DER SCHAFE. Arch. Protistenk. 42: [380]–399, illus. 1921.

[3] DOUWES, JOHN BRUGT. BIJDRAGE TOT DE KENNIS VAN ENKELE DARMPROTOZOEN DER HUISDIEREN IN HET BIJZONDER BIJ SCHAAP EN VARKEN. 62 pp. Utrecht. 1921.

[4] YAKIMOFF, W. L., GALOUZO, I. G., RASTEGAÏEFF, E. F., MIZKEWITSCH, W. J., and TOLSTOFF, A. N. UEBER DIE DARMKOKZIDIOSE DER SCHAFE IN RUSSLAND (U. S. S. R.). Berlin Tierärztl. Wchnschr. 42: 877–881. 1926.

[5] CARRÉ, H. LA COCCIDIOSE DU MOUTON. Rec. Méd. Vét. 104: 530–539. 1928.

than those of other species from sheep. An attempt was made in the experimental procedures to duplicate conditions that actually exist in the fold and the pasture, in order to make the results directly applicable to conditions existing in the natural environment.

MATERIALS AND METHODS

Freshly discharged oöcysts of *Eimeria arloingi* were obtained from fecal pellets from several 4- to 8-week-old lambs kept near the laboratory of the Zoological Division of the Bureau of Animal Industry, at Beltsville, Md., where this investigation was conducted. Almost daily fecal examinations were made on a total of nine lambs, four during April 1937, and five late in March and early in April 1938. The lambs, with their mothers, were kept in an enclosure 50 feet long by 20 feet wide, and their milk diet was supplemented with water, grain, and hay. Tap water was supplied in a tub, the grain was fed in an open trough upon the ground, and the hay was scattered directly upon the ground. The lambs and ewes had access to the same feed and bedding straw, thereby providing excellent opportunities for the transmission of the infection to lambs from adult animals, the latter being latent carriers.

Few or no oöcysts were discharged by the lambs during the first 3 or 4 weeks after birth, but during the succeeding several weeks enormous numbers were passed. During this period of high oöcyst discharge there was usually a significantly high peak lasting several days during the fourth or fifth week after birth, followed by a lower grade, fluctuating discharge for the next few weeks. Fecal samples were obtained by isolating lambs inside individual, plank-floored pens until pellets were dropped. After release of the animals and collection of samples, each pen was carefully swept, locked, and dried until the next day. Fecal specimens containing oöcysts in sufficient numbers for sporulation experiments could be obtained at any time during the period of high oöcyst discharge.

In the fold and the pasture, discharged fecal pellets containing oöcysts may be subjected to the following conditions: (1) They may fall into running water, which washes the oöcysts relatively free from contaminating organic debris; (2) they may fall into stagnant water and form a layer of putrefying organic sediment on the bottom, in which the oöcysts are lodged; (3) they may fall upon and percolate into litter straw, hay, grass, or grain in feed boxes, where the oöcysts find favorable conditions of moisture for the preservation of viability for indefinite periods; or (4) they may fall in open places exposed directly to the drying action of sunshine and wind. The fecal pellets are exposed to temperature fluctuations, as well as to these natural environmental conditions. In laboratory tests, these possible environments for oöcysts were duplicated as nearly as possible, and each preparation was subjected to several temperatures within the range of those occurring in the natural environment.

To obtain oöcysts free from contaminating debris for sporulation tests in clean water, fresh fecal pellets were crushed in a mortar, mixed with water, and the mixture washed with more water through a 30-mesh sieve into glass containers for settling. After at least an hour of sedimentation, the supernatant fluid was poured off and 1-cc. lots of the sediment were thoroughly mixed with 14-cc. lots of 35-percent sugar solution in 15-cc. centrifuge tubes, which were then allowed to stand for another hour to permit flotation of the oöcysts.

By lifting off the center of the surface film of each tube with a wire loop, the oöcysts were removed from the tubes relatively free from fecal debris. These loopfuls containing oöcysts were placed upon the bottom of the spherical pits of 25- by 75-mm. culture slides, and clean tap water was added to fill the depressions. Oöcysts soon settled to the bottom of the pits and lay beneath about 2 mm. of clean water, with little contaminating debris to obstruct vision or cause putrefaction. The slides were labeled, placed inside moist chambers made by adding water to the floors of Petri dishes, covered, incubated at the desired temperatures, and removed at intervals to determine evidence of sporulation.

A putrefying organic environment was created by placing fecal sediment containing oöcysts in open dishes to depths of about 15 mm., and keeping it barely covered with water by periodic additions during incubation at different temperatures. At room and incubator temperatures it was also necessary to place preparations inside improvised moist chambers to prevent drying. To check for evidence of sporulation, quantities of the sediment were mixed with sugar solution and the floated oöcysts were transferred to slides with a wire loop and observed.

The ability of oöcysts to sporulate inside pellets in which the natural amount of fecal moisture is retained was tested by placing fresh pellets upon a layer of soaked filter paper inside closed Petri dishes and incubating at the desired temperatures. The wet paper insured a saturated atmosphere within the dish, thus preventing loss of fecal moisture. At intervals, oöcysts were removed from the pellets by the flotation method described and examined for evidence of sporulation.

To determine the ability of oöcysts to sporulate inside feces dried in air, fresh pellets were placed in open dishes, stored at the desired temperatures, and the oöcysts were removed at intervals for observation by the flotation method.

In preparations in which little or no sporulation occurred, viability tests were conducted at intervals to determine the longevity of oöcysts under given conditions. The criterion for viability was the ability of the oöcysts to develop normal sporocysts after periods in the unsporulated condition, when the protoplasm is in the form of a spherical sporont. The viability test consisted in isolation of oöcysts in clean water on culture slides and storage for at least 3 days at room temperature. If no sporulation occurred within that time, the oöcysts were considered as dead, since maximum normal sporulation of oöcysts of *Eimeria arloingi* takes place within 48 hours after removal into clean water at room temperature.

In the results reported in this paper, oöcysts were classified as follows: Those in which there was no sign of protoplasmic segmentation, as oöcysts with undivided sporont; those containing four-lobed sporonts, four spheres, pyramids, or oval sporoblasts, as oöcysts with the intermediate stages; those in which the protoplasm had split into two to eight or even more fragments of unequal size, as oöcysts showing abnormal segmentation; and those containing four refractile equal-sized, roughly spindle-shaped sporocysts, as oöcysts with complete sporocysts.

The longevity of oöcysts in the sporulated condition obviously cannot be determined by the method described in this paper. The

criterion for viability of sporulated oöcysts is the production of infection in a susceptible host, which is beyond the scope of the present study. It is believed, however, that the conclusions derived from these viability experiments with unsporulated oöcysts can be applied as well to those in the sporulated state, with the advantage in favor of the latter as a result of an additional protective shell about each sporocyst.

SPORULATION AND VIABILITY OF OÖCYSTS OF EIMERIA ARLOINGI IN SHALLOW TAP-WATER CULTURES

Oöcysts in shallow tap-water cultures kept at near-freezing temperature (0°–5° C.) sporulated slowly, 2 to 3 weeks elapsing before a significant amount of sporulation was noted (table 1). At room temperature (20°–25°) segmentation was rapid and normal, maximum sporulation occurring within 48 hours. Since these cultures gave uniformly higher percentages of sporulation than any others in the series, the conditions were considered to be optimum for sporulation and became the basis for viability tests. Oöcysts in cultures kept at 32° segmented at an accelerated rate but in an abnormal manner. There were evidences of protoplasmic splitting in more than half of them at the end of 24 hours. At 72 hours, which is an interval sufficient for the production of four equal-sized spores in each oöcyst in cultures kept at room temperature, the protoplasm of most of the oöcysts had segmented into two to eight, occasionally more, fragments of unequal size and shape. There was no sign of sporulation in oöcysts kept at 40° C. for as long as 10 days.

TABLE 1.—*Sporulation and viability of oöcysts of Eimeria arloingi beneath 2 mm. of clean tap water*

Experiment No.	Temperature of preparation during sporulation test	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test					Results of viability test [1]			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts showing abnormal segmentation	Oöcysts with complete sporocysts	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts
	° C.			Days	No.	Pct.	Pct.	Pct.	Pct.	No.	Pct.	Pct.	Pct.
1	0–5	1	Apr. 13, 1937	7	40	100	0	0	0	------	------	------	------
				13	111	59	23	0	18	------	------	------	------
		2	Mar. 30, 1938	12	70	100	0	0	0	------	------	------	------
				16	66	76	21	0	3	------	------	------	------
				23	56	7	63	0	30	------	------	------	------
2	20–25	1	Apr. 19, 1937	5	694	4	0	0	96	------	------	------	------
				Hrs.									
		2	May 27, 1937	26	121	50	24	0	26	------	------	------	------
				47	129	6	6	0	88	------	------	------	------
		3	Mar. 30, 1938	24	90	58	42	0	0	------	------	------	------
				30	95	19	38	0	43	------	------	------	------
				48	88	3	3	0	94	------	------	------	------
3	32	1	Apr. 5, 1938	24	108	47	7	26	20	------	------	------	------
				41	107	18	7	54	21	------	------	------	------
				72	126	10	2	73	15	------	------	------	------
				Days									
4	40	1	Mar. 30, 1938	1	100	100	0	0	0	79	34	63	3
		2	Apr. 1, 1938	3	300	100	0	0	0	274	60	40	0
		3	Apr. 5, 1938	10	100	100	0	0	0	100	100	0	0

[1] Determined after 3 or more days beneath 2 mm. of clean water at 20°–25° C.

The results of the viability test, also given in table 1, showed that practically all the oöcysts kept at 40° C. in shallow tap-water cultures were killed by exposure for 2 days to that temperature and that all were killed in cultures exposed 3 and 10 days.

Failure to sporulate normally or at all at the higher temperatures is attributed to insufficiency of oxygen, since the oxygen-holding capacity of water diminishes with increase in temperature.

IN PUTREFYING FECAL SEDIMENT

At near-freezing temperature (0°–5° C.), all oöcysts in fecal sediment covered with shallow water remained unsporulated after 10 months' exposure (table 2); at room temperature (20°–25°), all were unsporulated after 1 year; and at 40°, there was no sporulation in 17 days. The protoplasm in these oöcysts retained the form of spherical sporonts, which gradually diminished in size and showed progressive disintegration as preparations aged.

TABLE 2.—*Sporulation and viability of oöcysts of Eimeria arloingi in fecal sediment covered with shallow water*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test		Results of viability test [1]			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts
	°C.			Days	Number	Percent	Number	Percent	Percent	Percent
1	0–5	1	May 26, 1937	50	100	100	200	4	0	96
				201	100	100	223	18	0	82
				314	200	100	123	19	62	19
		2	Sept. 22, 1937	113	50	100	50	4	0	96
2	20–25	1	Apr. 12, 1937	368	100	100	100	0	100	0
		2	Apr. 16, 1937	33	89	100	40	48	0	52
		3	May 25, 1937	127	100	100	38	100	0	0
		4	Apr. 1, 1938	19	100	100	54	6	0	94
		5	Apr. 11, 1938	7	100	100	100	4	0	96
3	40	1	May 25, 1937	17	212	100	156	100	0	0
		2	Mar. 31, 1938	4	100	100	46	78	22	0

[1] Determined after 3 or more days beneath 2 mm. of clean water at 20°–25° C.

The viability test showed the approximate point at which death of the protoplasm took place. Oöcysts kept in fecal sediment at near-freezing temperature lost viability slowly and gradually, as seen in table 2. There was no perceptible loss in ability to sporulate in nearly 4 months, after which oöcysts gradually died until only about one-fifth of them were viable at the end of 10 months. At room temperature the loss of viability was more rapid. Approximately half of the oöcysts remained viable at 1 month, whereas all were killed within 4 months. All oöcysts in preparations incubated at 40° C. were killed within 4 days.

The absence of sporulation in the oöcysts is attributed to the oxygen insufficiency resulting from putrefaction rather than to toxic products of the process. The fact that about 20 percent of these unsporulated oöcysts were still viable after 10 months at near-freezing temperature indicates that oöcysts may accumulate in wet situations in the outside environment and survive for many months during colder parts of the year and that they retain the ability to sporulate

and become infective to susceptible hosts when appropriate conditions are restored. This ability of oöcysts to live for long periods in wet situations in colder weather and the marked lethal influence of high temperatures suggest the advisability of selecting sunny, well-drained terrain for sheep lots and pasture and of eliminating sites that favor the accumulation of waste water.

INSIDE FECAL PELLETS IN WHICH NATURAL MOISTURE WAS PRESERVED

At near-freezing temperature (0°–5° C.), oöcysts inside fecal pellets in which natural moisture was preserved sporulated slowly, only about one-fifth of them showing signs of segmentation within 10 months (table 3). Sporulation was rapid at room temperature (20°–25°), only 3 days being required for the majority of the oöcysts to develop mature sporocysts. There was no sign of sporulation within 4 days in preparations incubated at 40°.

TABLE 3.—*Sporulation and viability of oöcysts of Eimeria arloingi inside fecal pellets in which natural moisture was preserved*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test				Results of viability test [1]			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts with complete sporocysts	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts
	°C.			*Days*	*Number*	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*
1	0–5	1	May 26, 1937	10	100	100	0	0	106	6	0	94
				126	117	86	12	2	100	7	0	93
		2	Apr. 1, 1938	314	173	82	15	3	155	19	57	24
				10	100	100	0	0	------	------	------	------
2	20–25	1	Apr. 19, 1937	3	577	29	11	60	------	------	------	------
				9	744	20	0	80	------	------	------	------
				36	103	3	0	97	------	------	------	------
		2	Mar. 31, 1938	1	163	92	8	0	------	------	------	------
				2	127	45	13	42	------	------	------	------
				4	109	46	4	50	------	------	------	------
		3	Apr. 11, 1938	3	98	4	1	95	------	------	------	------
				7	100	5	0	95	------	------	------	------
		4	----do----	3	100	9	1	90	------	------	------	------
				7	100	0	0	100	------	------	------	------
3	40	1	Mar. 31, 1938	4	100	100	0	0	100	98	2	0

[1] Determined after 3 or more days beneath 2 mm. of clean water at 20°–25° C.

The results of the viability test (table 3) showed that there was no appreciable loss of ability to sporulate in oöcysts inside natural pellets kept for 4 months at near freezing temperature, but during the next 6 months three-fourths of them lost viability. At 40° C., oöcysts inside the pellets were rapidly killed, being unable to sporulate after an exposure of 4 days.

These observations suggest the desirability of frequent changes of bedding straw and also of feeding from racks and elevated troughs rather than off the ground in order to eliminate environmental conditions favorable for the preservation of the natural moisture in fecal pellets and to reduce the chances of feed being contaminated with sporulated oöcysts.

INSIDE FECAL PELLETS DRIED IN AIR

Inside fecal pellets dried at room temperature (20°–25° C.), sporulation occurred before total desiccation. A majority of the oöcysts sporulated within 3 days (table 4) before the oöcyst walls were so extensively wrinkled and blackened that it was impossible to distinguish the contents. After 2 days of drying at 40°, oöcyst walls were extremely wrinkled, distorted, and blackened, but one-sixth of the oöcysts contained what appeared to be normal sporocysts.

TABLE 4.—*Sporulation of oöcysts of Eimeria arloingi inside fecal pellets dried in air*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of test	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts in which wrinkling hid contents	Oöcysts with recognizable sporocysts
	° *C.*			*Days*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
1	20-25	1	Apr. 5, 1938	1	100	100	0	0	0
				2	93	3	5	38	54
				6	100	0	0	100	0
		2	Apr. 11, 1938	3	67	10	0	10	80
				7	54	0	0	93	7
2	40	1	Apr. 5, 1938	2	104	39	2	43	16
				6	100	0	0	100	0

The data of these experiments indicate that drying is probably fatal to oöcysts within several days or a few weeks as a result of the loss of water necessary for the vital activities of protoplasm. This observation again emphasized the importance of dry, well-drained land for sheep lots and pasture.

SUMMARY

The results of sporulation and viability experiments on oöcysts of *Eimeria arloingi* from domestic sheep are presented. These experiments demonstrated several facts of practical importance in the control of ovine coccidiosis.

Oöcysts covered with 2 mm. of clean water sporulated slowly at near-freezing temperature (0°–5° C.) and normally and rapidly at room temperature (20°–25°). At 32° sporulation was accelerated but segmentation was abnormal, and at 40° the oöcysts failed to show signs of sporulation. The viability test showed that the unsporulated oöcysts in the cultures incubated at 40° were killed when exposed for 3 days. Abnormal segmentation and failure to sporulate at the higher temperatures were apparently due to insufficiency of oxygen.

There was no indication of sporulation at near-freezing, room, or incubator temperatures in oöcysts kept in fecal sediment covered with a shallow layer of water. Failure to sporulate under these conditions was attributed to lack of oxygen resulting from putrefaction. About 20 percent of the unsporulated oöcysts were still viable after 10 months at near-freezing temperature, indicating that oöcysts may accumulate in wet situations in the outside environment and survive for many months during the colder parts of the year, and that they retain the ability to sporulate and become infective to susceptible hosts when appropriate conditions are restored.

Oöcysts inside fecal pellets in which moisture was preserved showed little sporulation at near-freezing temperature, abundant and rapid sporulation at room temperature, and failure to sporulate at 40° C. These observations indicate that simple, sanitary measures in lamb raising, such as frequent changes of bedding straw and feeding from elevated racks and troughs, would greatly reduce the chances of contamination of the feed with resultant infection.

When fecal pellets were dried in air at room temperature and at 40° C., many of the contained oöcysts sporulated before desiccation had produced such extensive wrinkling and shrinkage that recognition of contents was impossible. It was concluded that drying was probably fatal to oöcysts within several days or a few weeks, emphasizing the importance of dry, well-drained land for sheep lots and pasture.